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Leukotrienes, Prostaglandins, and Granulocyte Accumulation in Cerebral Ischemia

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Introduction

Granulocytes accumulate during the early hours of the postischemic period in a canine model of multifocal ischemia induced by air embolism [9]. This accumulation could theoretically aggravate and extend the initial tissue injury through one of several mechanisms. Granulocytes synthesize arachidonic acid metabolities such as prostaglandins, leukotrienes, and hydroxy acids capable of producing local vasoconstriction and increased microvascular permeabiliity [1, 5, 32]. They also produce superoxide and other free radical products, release hydrolytic enzymes from intracellular granules, and synthesize platelet activating factor [21, 22, 33, 34].

The arachidonic acid cascade has a potential influence on granulocyte accumulation and the evolution of tissue damage in a zone of CNS ischemic injury. Leukotrienes and hydroxy acids, products of the lipoxygenase pathway of arachidonic acid metabolism, are potent granulocyte chemotaxins [1, 5, 20, 31]. The cyclooxygenase pathway products, thromboxane A_2 and PGI_2, are thought to influence vascular tone and platelet aggregation [6, 7].

Based on the above, it would seem reasonable to predict that modulation of arachidonic acid metabolism would affect the accumulation of granulocytes in ischemic brain, cerebral blood flow in the injury zone, and postischemic recovery of neuronal function as indicated by the cortical somatosensory evoked response (CSER). Specifically, cyclooxygenase inhibition (indomethacin) combined with PGI_2 and heparin should augment the CSER and eliminate "neuron-disabling" blood flows after ischemia as previously observed [10]. Granulocyte accumulation under these conditions should be more pronounced due to the increased formation rate of chemotactic products of the lipoxygenase pathway that accompanies cyclooxygenase inhibition [13, 14, 16, 24]. Substitution of dual cyclooxygenase and lipoxygenase inhibition (BW 755C) for the

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indomethacin should reduce the accumulation of granulocytes and, by restricting access of these potentially harmful cells to the injury zone, foster a relatively greater CSER recovery. These predictions were tested in the canine model of focal ischemia induced by incremental air embolism [9, 10].

Methods

Eighteen conditioned, male mongrel dogs (9–15 kg) were premedicated with subcutaneous xylaxine (1.1 mg/kg) and atropine (0.05 mg/kg). Surgical anesthesia was induced with α-chloralose (80 mg/kg) and maintained with doses of 20 mg/kg as needed. Anesthetized animals were mechanically ventilated and monitored for mean aortic blood pressure (MAP), temperature, hematocrit, arterial blood gases, and end tidal CO_2 and O_2 tensions, and prepared for recording of CSER. Rectal temperature was maintained at 36°–38.5°C with heating pads and warm water bottles. Two catheters placed in the right femoral artery were used for rapid blood sampling during the CBF study as previously described [10]. A right ventricular catheter was placed in the right femoral artery for the rapid termination of the CBF study with saturated potassium chloride. A catheter placed in the aorta from the left femoral artery was connected to a strain gauge to monitor arterial blood pressure and a right femoral venous catheter was advanced into the inferior vena cava for infusion of solutions throughout the experiments. A polyethylene catheter was inserted into the right cephalic vein for the induction of anesthesia and the infusion of PGI_2 when appropriate. The right internal carotid artery was exposed and catheterized with PE-50 tubing. Electrocardiographic leads were connected to monitor heart rate and rhythm.

Prior to ischemia, 102 ml of blood was collected from a femoral artery catheter and placed in 18 ml of anticoagulant citrate dextrose solution (ACD-Formula A, Fenwall Laboratories, Deerfield, IL). Ringer's lactate solution (100 ml) was injected immediately after blood sampling. After dilution of the blood with dextran 500 (6% in saline, 1 part dextran to 10 parts blood), the mixture was allowed to sediment for 30–45 min or until the erythrocytes occupied just less than half the height of the tube. The supernatant containing plasma, leukocytes, and platelets was then washed and centrifuged at 250 × g for 10 min, and the leukocyte-rich pellet was loaded into a Beckman, JE-4B elutriator at a flow of 5 ml/min. The remaining plasma proteins, platelets, erythrocytes, and mononuclear cells were removed by gradually increasing the flow rate through the separation chamber until only the granulocytes remained. The granulocyte suspension was spun at 185 × g for 10 min and the pellet obtained was resuspended in 3 ml saline and incubated with 1 mCi ^{111}In-oxine. Autologous, ^{111}In-labeled granulocytes were reinfused 5 min prior to the onset of ischemia. Purity of the recovered granulocytes was assessed by cell morphology, and viability was determined by trypan blue exclusion. The percent of ^{111}In-labeled granulocytes circulating at time points after initial infusion, and the granulocyte labeling efficiency were determined exactly as in our prior study [9].

The dogs were placed in a Kopf sterotaxic apparatus and prepared for the recording of CSER. After surgical exposure of the skull, stainless steel screw electrodes were positioned over the right sensorimotor cortex and the nasal bones (reference electrode). Electrode impedances were < 3 kohm throughout the experiments. Stimulating electrodes were positioned in the left upper foreleg such that the median nerve was between them. A square wave stimulus of 300 μs at a rate of 1.1/s was led to the stimulating electrodes from a Nicolet Model NIC-1003A current stimulator. The stimulus strength was adjusted to cause a maximal CSER over the contralateral sensorimotor cortex. Potentials from the recording electrodes were led to a Nicolet Model HGA-200A physiological amplifier and the output was led to a Nicolet Model NIC-CA 1000 clinical averager. The output was displayed (Hewlett Packard Model 1340A X-Y) and recorded (Hewlett Packard Model 701, CBX-Y). Evoked potentials ($n = 64$) were averaged to obtain each recorded response.

Focal ischemia was induced in the right hemisphere by infusing 50 μl of air into the right internal carotid artery. CSERs generated by stimulation of the left median nerve were obtained every 2 min during a 60-min ischemic period. Intermittent boluses of 20–50 μl of air were injected into the right internal carotid artery to maintain suppression of the P1–N1 amplitude of the CSER at 10%–20% of its baseline value. At the end of the 60-min ischemic suppression, seven animals received no therapy and 11 animals were treated (Table 1). Treatment was initiated immediately following the 60-min ischemic period. CSER measurements were obtained every 10 min in all animals throughout the 4-h recovery, and the percent recovery of the P1–N1 amplitude relative to baseline was recorded. Control groups using PGI_2, heparin, or both combined were previously shown to have no effect on CSER or CBF recovery in this model [10].

PGI_2 (Upjohn Co., Kalamazoo, MI) was prepared as a 25 μg/ml solution in 0.1 M tris-HCl/0.15 M NaCl at pH 8.5 and was continuously infused throughout the recovery period at 90–140 ng/kg per minute. Indomethacin (Indocin, gift of Merck, Sharp, Dohme, West Point, PA) was prepared shortly before infusion as a 2 mg/ml solution in 0.1 M tris-HCl/0.15 M NaCl at pH 8.5. Indomethacin was administered as a single 4 mg/kg bolus immediately after the initiation of PGI_2 infusion. BW 755C, 3-amino-1-*m* (trifluoromethyl)-phenyl-2-pyrazoline (Wellcome Research Laboratories, United Kingdom) was freshly prepared as a 10 mg/ml solution in distilled water. This solution was infused as a single 10 mg/kg bolus given over 10–15 min. The BW 755C was protected from light during infusion. More rapid infusion of this solution produced

Table 1. Definition of experimental groups

Group	No. of animals	Indomethacin (4 mg/kg)	BW 755C (10 mg/kg	PGI_2 (90–140 ng/kg/min)	Heparin (300 IU/kg)
I	7	0	0	0	0
II	6	+	0	+	+
III	5	0	+	+	+

hemolysis. Heparin (American Biologics Co., Philadelphia, PA) was given as a single bolus (300 IU/kg) immediately after indomethacin or during BW 755C administration.

At the end of the 4-h recovery period, a 1-min ^{14}C-iodoantipyrine, autoradiographic CBF study was performed as previously described [10]. After euthanasia with saturated KCl, the brain was removed and immediately frozen at $-50°$ to $-60°$ C in liquid freon suspended over liquid nitrogen. The brain was later divided coronally into three segments, each containing symmetrical portions of the right and left hemispheres. The anterior portion contained the head of the caudate nucleus, the middle portion contained the thalamus, and the posterior portion contained the lateral ventricle and the adjacent hippocampal formation. Each segment was then mounted and 20-μm sections were cut on a cryogenic microtome for the autoradiographic determination of CBF. Immediately after sectioning, samples of the cortex were excised from homologous watershed areas of the superolateral right and left hemispheres at the level of each segment. The right hemisphere constituted the injured side and the left hemisphere constituted the noninjured side. The samples were weighed and counted (LKB Wallac CompuGamma counter, Turku, Finland) with a 125–500 keV energy window. Radioactivity, expressed as cmp/g tissue, provided an index of granulocyte accumulation in the three brain segments and a right–left difference (cmp/g) was calculated for each segment of each animal. A mean, hemispheric, right–left difference was determined for each animal using the mean of the three segmental right–left differences.

Results

Hematocrit (Hct), pH, $PaCO_2$, and rectal temperature did not differ significantly between the groups at any of the three sampling times. The amounts of air required to produce an ischemic reduction of CSER to 10%–20% of its baseline value during the 60-min ischemic interval were 0.22 ± 0.11 cc (mean ± SD), 0.15 ± 0.07 cc, and 0.22 ± 0.18 cc in groups I–III, respectively. These volumes did not differ significantly. The percent of CSER readings during ischemia below 20% of the initial amplitude was also used as an index of the severity of ischemia. Values for groups I–III were 72 ± 12, 71 ± 15, and 59 ± 17 (mean ± SD), respectively. There was no significant difference between the groups. The final CSER (4 h) expressed as the percent of baseline P1–N1 amplitude for the three experimental groups is depicted in Table 2. Although

Table 2. Percent recovery of CSER after 4 h of reperfusion (mean ± SD)

Group	Percent recovery	Significance
I	21 ± 5	I
II	38 ± 14	NS $P<0.01$
III	47 ± 8	II III
		NS

Table 3. Right-left hemispheric differences in indium 111-labeled granulocyte activity

Group	Percent recovery	Significance
I	3298 ± 2019	I
II	3807 ± 1629	NS $P<0.01$
III	2828 ± 1878	II III
		NS

treatment with indomethacin, PGI_2, and heparin (group II) did not produce significant recovery of the final CSER amplitude when compared with untreated animals (group I) (38.0% ± 13.6% vs 21.0% ± 4.6%, mean ± SEM), this can be stated with a power of only 0.33 (type II error estimation). A previous randomized, blinded comparison of this treatment regimen and untreated controls in this model demonstrated significant recovery of CSER amplitude after 1 h of reperfusion in treated animals [10]. Animals treated with BW 755C, PGI_2, and heparin (group III) recovered 47% ± 8% of their baseline CSER which differed from untreated animals at $P<0.01$.

The efficiency of granulocyte labeling was 97% ± 2% (mean ± SD), and the percentage of ^{111}In activity in the injected granulocyte suspensions that was not in cells was 1.8% ± 1.1%. The purity and viability of the granulocytes averaged greater than 95%. The mean right − left hemispheric differences in ^{111}In activity after 4 h of postischemic reperfusion (cpm/g, mean ± SD) are shown in Table 3. Mean right − left hemispheric differences in ^{111}In activity did not differ significantly between the three groups. Based on the sample size and the standard deviation of our samples, a 50% change in granulocyte accumulation could have been shown with a power of 0.50.

Average CBF rates in gray matter and white matter of damaged and nondamaged hemispheres 4 h after ischemia did not differ between the untreated and treated groups. Neuron-disabling blood flows were defined as 15 ml/100 g per minute or less in gray matter, and 6 ml/100 g per minute or less in white matter [10]. Two of seven untreated animals had flows in this range. As in our previous study, indomethacin, PGI_2, and heparin completely eliminated the occurrence of neuron-disabling blood flows at 4 h postischemia. BW 755C, PGI_2, and heparin also eliminated any incidence of neuron-disabling blood flows.

Discussion

The principal findings of these experiments were that indomethacin, PGI_2, and heparin did not significantly ameliorate CSER recovery at 4 h of postischemic reperfusion, although neuron-disabling blood flows were eliminated, and granulocyte accumulation in this group did not differ significantly from untreated animals. BW 755C, PGI_2, and heparin conferred a CSER recovery that was sustained for 4 h and also eliminated neuron-disabling blood flows, but did not significantly reduce the accumulation of granulocytes.

Several questions are raised by these results. What is the relationship between neuron-disabling blood flows and CSER recovery after 4 h of postischemic reperfusion? What is the relationship between lipoxygenase-derived chemotaxins and granulocyte accumulation in this model? What is the relationship between granulocyte accumulation and neuronal recovery in the injury zone as indicated by the CSER?

The presence of "neuron-disabling" flows correlates with poor CSER recovery in this model [10], but it is not a necessary condition for poor recovery because only two of seven untreated animals had flows in this range. The "neuron-disabling" flows do not need to involve neuroanatomical pathways presumed to subserve the CSER to augur a poor electrophysiological recovery. A low flow in any of the measured neuroanatomical areas is associated with a relatively poor outcome. This suggests that postischemic hypoperfusion is not the principal cause of neuronal injury or impaired neuronal recovery in the postischemic period. Instead, the hypoperfusion appears to be but one manifestation of a more fundamental process that is deleterious to the restoration of neuronal function in a postischemic injury zone. Light and electron microscopic analysis of the morphological changes in neurons subjected to 1–2 h of global brain ischemia without any reperfusion reveals a slow evolution of chromatin clumping and cell swelling which is homogeneous and uniform [18]. In contrast, brains subjected to a similar total time of exposure but permitted to undergo a period of reperfusion after a 10- to 15-min period of global ischemia reveal a strikingly different cytopathological picture [17]. Cellular morphology becomes heterogeneous, with numerous neurons showing only chromatin clumping, nucleolar condensation, and a breakdown of polysomes. This has been interpreted as representing a reversal of some of the changes produced by complete global ischemia. Other cells, ranging up to about 25% of the total population, show severe cell change with marked shrinkage and few identifiable subcellular structures in the electron-dense cytoplasm. These neurons are found primarily in cortical layers 3, 5, and 6, zones of selective vulnerability. Some level of postischemic microvascular perfusion seems required for the development of these changes, implicating factors derived from blood. Activation of a multifactorial process through blood–damaged tissue interaction would be one plausible explanation for these findings [11]. Instead of leading to tissue damage primarily through interference with oxygen and substrate delivery, and impaired clearance of metabolic wastes due to microcirculatory shutdown, the critical effect of the blood–damaged tissue interaction might be the production of mediators of direct tissue injury. Focal ischemia, as the proximate cause of the tissue damage, is rendered unlikely because microcirculatory shutdown taken to its ultimate degree would create a focus resembling an area of complete cerebral ischemia as in the permanent global ischemia experiments. The observed cytopathology is thus incompatible with this mechanism.

Postischemic hypoperfusion that is disproportionately lower than regional cerebral glucose utilization does occur [19, 27] and could exacerbate the interaction between blood and damaged tissue [11, 12]. Postischemic impairment of microcirculatory perfusion has been well studied after temporary focal ischemia [15], and the differential vulnerability of neighboring neurons to the same

perfusion microenvironment has been documented [29]. Progressive impairment of microcirculatory flow has been noted after permanent middle cerebral artery branch occlusion [23], and focal hypoperfusion has been observed in brain regions subjected to a metabolic insult in the absence of any primary occlusion of vessels [8]. Thus, temporary or permanent vessel occlusion as well as nonischemic causes of brain injury can lead to microcirculatory hypoperfusion. Such hypoperfusion probably exacerbates the interaction between blood and damaged tissue rather than serving as the principal mediator of further neuronal damage. Prime candidates for the principal mediators of tissue damage which could be derived from blood-damaged tissue interaction would include leukotrienes, prostaglandins, thromboxanes, free radicals, leukocyte and platelet accumulation with elaboration of their diverse mediators, and activation of the complement, coagulation, and fibrinolytic systems [9, 11].

The failure of BW 755C to inhibit granulocyte accumulation significantly when given after 60 min of focal ischemia can be explained in at least two ways: either inhibition of the lipoxygenase pathway occurred too late to prevent early granulocyte accumulation, or lipoxygenase-derived products are not the controlling chemotaxins involved in this process. In this model of incomplete ischemia, oxygen-dependent lipoxygenase product formation very likely occurs during the 60-min ischemia period prior to the initiation of treatment. Thus, inhibition of lipoxygenase product formation may have occurred too late to block rapid granulocyte accumulation effectively. However, previous kinetic studies in our laboratory demonstrated that granulocyte accumulation did not occur during ischemia and was not significant until 1–4 h after ischemia [9]. To explain the failure of BW 755C to inhibit rapid granulocyte accumulation when administered after ischemia and still invoke a major role for lipoxygenase-derived products in this process, a gradient of lipoxygenase products would have to have formed during the initial 60-min ischemia period with subsequent gradual granulocyte accumulation over the first 4 h of reperfusion. The failure of BW 755C pretreatment (unpublished data) to inhibit early postischemic granulocyte accumulation argues against this possibility. Also, PGI_2, indomethacin, and heparin, which should increase the production of lipoxygenase-derived leukotrienes and hydroxy acids [13, 14, 16, 24], failed to exacerbate granulocyte accumulation in this series. These data suggest that lipoxygenase products are not the controlling chemotaxins responsible for early postischemic granulocyte accumulation in the brain. Alternative chemotaxins that may be involved include components of the complement system, kallikrein, plasminogen activator, fragments of the fibrinolytic pathway, superoxide radical, and possibly other substances [2, 26, 30].

Granulocytes possess cellular machinery capable of inflicting tissue injury during postischemic reperfusion [1, 5, 21, 22, 33, 34]. Accumulation of these cells during early postischemic reperfusion supports, but does not prove, a role for their participation in postischemic reperfusion injury. Romson et al. [28] and Mullane et al. [25] reported results from models of myocardial infarction that strongly support a role for granulocytes in this process. In these two studies, neutropenic animals demonstrated significant reductions in myocardial infarct size when compared with control animals after 4 h of reperfusion. Our data

suggest that granulocytes accumulate in proportion to the severity of ischemia and, therefore, may play a role in determining the ultimate infarct size in this model. There is a need to evaluate further the effect of interventions which modify granulocyte accumulation and function on postischemic neuronal recovery. Although BW 755C, PGI_2, and heparin did not alter early postischemic granulocyte accumulation, it is possible that the therapeutic effect on postischemic CSER recovery and reperfusion involves modulation of granulocyte function. For example, PGI_2 rapidly and reversibly inhibits particulate stimuli and FMLP-induced superoxide production in human neutrophils and inhibits neutrophil granule release in vitro [3, 4].

References

1. Dahlen SE, Jakob B, Hedqvist P, Arfors KE, Hammarstrom S, Lindgren JA, Samuelsson B (1981) Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules: in vivo effects with relevance to the acute inflammatory response. Proc Natl Acad Sci USA 78(6):3887–3891
2. Del Principe D, Menichelli A, Galli E, Persiani M, Perlini R, D'Arcangelo C, Businco L, Rossi P (1982) Superoxide-dependent chemotactic activity for PMNs derived from opsonized zymosan-stimulated human platelets. Pediatr Res 16:1000–1003
3. Fantone JC, Marasco WA, Eligas LJ, Ward PA (1984) Stimulus specificity of prostaglandin inhibition of rabbit polymorphonuclear leukocyte lysosomal enzyme release and superoxide anion production. Am J Pathol 115:9–16
4. Fantone JC, Kinnes DA (1983) Prostaglandin E_1 and prostaglandin I_2 modulation of superoxide production by human neutrophils. Biochem Biophys Res Commun 113(2):506–512
5. Gimbrone MA, Brock AF, Scafer AI (1984) Leukotrine B_4stimulates polymorphonuclear leukocyte adhesion to cultured vascular endothelial cells. J Clin Invest 74:1552–1555
6. Gorman RR (1978) Prostaglandins, thromboxanes, and prostacyclin. In: Rickenberg HB (ed) International review of biochemistry. Biochemistry and mode of action of hormones II, Volume 20. Baltimore, University Park Press, pp 81–107
7. Gryglewski RJ, Bunting S, Moncade S, Flower RJ, Vane JR (1976) Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin X) which they make from prostaglandin endoperoxides. Prostaglandins 12:685–713
8. Hakim AM (1986) Effect of thiamine deficiency and its reversal on cerebral blood flow in the rat. Observations on the phenomena of hyperperfusion, "no reflow," and delayed hypoperfusion. J Cereb Blood Flow Metab 6:79–85
9. Hallenbeck JM, Dutka AJ, Tanishima T, Kochanek PM, Kumaroo KK, Thompson CB, Obrenovitch TP, Contreras TJ (1986) Polymorphonuclear leukocyte accumulation in brain regions with low blood flow during the early post-ischemic period. Stroke 17:246–253
10. Hallenbeck JM, Leitch DR, Dutka AJ, Greenbaum LJ, McKee AE (1982) Prostaglandin I_2, indomethacin, and heparin promote post-ischemic neuronal recovery in dogs. Ann Neurol 12(2):45–156
11. Hallenbeck JM, Leitch DR, Dutka AJ, Greenbaum LJ Jr (1982) The amount of circumscribed brain edema and the degree of post-ischemic neuronal recovery do not correlate well. Stroke 13:797–804
12. Hallenbeck JM (1977) Prevention of post-ischemic impairment of microvascular perfusion. Neurology (Minneapolis) 27:3–10
13. Hamberg M, Svenson J, Samuelsson B (1974) Prostaglandin endoperoxides. A new concept concerning the mode of action and release of prostaglandins. Proc Natl Acad Sci USA 71:3824
14. Hambert M (1976) On the formation of thromboxane B_2 and I_2-hydroxy-*r*, 8, 10, 14-eicosatetraenoic acid in tissues from the guinea pig. Biochem Biophys Acta 431:651

15. Heiss WD, Rosner G (1983) Functional recovery of cortical neurons as related to degree and duration of ischemia. Ann Neurol 14:294–301
16. Higgs GA, Mugridge KG (1983) Inhibition of mononuclear leukocyte accumulation by the arachidonic acid lipoxygenase inhibitor BW 755C. Adv Prostaglandin Thromboxane Res 12:19
17. Jenkins LW, Povlishock JT, Lewelt W, Miller JD, Becker DP (1981) The role of post-ischemic recirculation in the development of ischemic neuronal injury following complete cerebral ischemia. Acta Neuropathol (Berl) 55:205–220
18. Kalimo H, Garcia JH, Kamijyo Y, Tanaka J, Trump BF (1977) The ultrastructure of "brain death" II. Electron microscopy of feline cortex after complete ischemia. Virchows Arch B Cell Path 25:207–220
19. Levy D, Van Uitert R, Pike C (1979) Delayed postischemic hypoperfusion: a potentially damaging consequence of stroke. Neurology (NY) 29:1245–1252
20. Lewis RA, Austen KF (1981) Mediation of local hemostasis and inflammation by leukotrienes and other mast cell-dependent compounds. Nature 293:103–108
21. Lynch JM, Lotner GZ, Betz SJ, Henson PM (1979) The release of a platelet activating factor by stimulated rabbit neutrophils. J Immunol 123(1):1219–1266
22. McCord JM, Roy RS (1982) The pathophysiology of superoxides: roles in inflammation and ischemia. Can J Physiol Pharmacol 60:1346–1352
23. Meyer FB, Anderson RE, Sundt TM, Yaksh TL (1986) Intracellular brain pH, indicator tissue perfusion, electroencephalography, and histology in severe and moderate focal cortical ischemia in the rabbit. J Cereb Blood Flow Metab 6:71–78
24. Mitchell HW (1982) Effect of ETYA and BW 755C on arachidonate-induced contraction in the guinea-pig isolated trachea. Br J Pharmacol 76:527
25. Mullane KM, Read N, Salmon JA, Moncada S (1984) Role of leukocytes in acute myocardial infarction in anesthestized dogs: relationship to myocardial salvage by anti-inflammatory drugs. J Pharmacol Exp Ther 228(2):510–521
26. O'Flaherty JT, Ward PA (1979) Chemotactic factors and the neutrophil. Seminars in Hematology 16(2):163–174
27. Pulsinelli WA, Levy DE, Duffy TE (1982) Regional cerebral blood flow and glucose metabolism following transient forebrain ischemia. Ann Neurol 11:449–509
28. Romson JL, Hook BG, Kunkel SL, Abrams GD, Schock MA, Lucchesi BR (1983) Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. Circulation 67(5):1016–1023
29. Rosner G, Graf R, Kataoka K, Heiss WD (1986) Selective functional vulnerability of cortical neurons following transient MCA-occlusion in the cat. Stroke 17:76–82
30. Rossen RD, Swain JL, Michael LH, Weakley S, Gianni E, Entman ML (1985) Selective accumulation of the first component of complement and leukocytes in ischemic canine heart muscles. Circ Res 57:119–130
31. Samuelsson B (1983) Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. Science 220:568–575
32. Stenson WF, Parker CW (1979) Metabolism of arachidonic acid in ionophore-stimulated neutrophils. J Clin Invest 64:1457–1465
33. Tate RM, Repine JE (1983) Neutrophils and the adult respiratory distress syndrome. Am Rev Respir Dis 128:552–559
34. Weissman G, Smolen JE, Korchak HM (1980) Release of inflammatory mediators from stimulated neutrophils. N Engl J Med 303–327


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